REMARKS

With this amendment, claims 1-6, 11, 13-18, 23 and 25-37 are cancelled. Pending claims are claims 7-10, 12, 19-23, 24 and 38-41.

With this amendment, claims 1-6, 11, 13-18, 23 and 25-37 are cancelled. Pending claims are claims 7-10, 12, 19-22, 24 and 38-41. New claim 41 depends from claim 7 and recites that hydrolysis results in a growth advantage of the cell compared to cells which do not have the construct, as discussed, for example, in the specification at page 4, lines 19-25, page 5 lines 9-16, page 26 line 24 – page 27 line 2, page 28 line 21 – page 29 line 18 and Example 6.

The Applicant appreciates the interview with the Examiner on September 27, in which the Applicant discussed presentation of the claims of this amendment. The claims, as amended, are directed to plant cells, and their progeny (as referenced, for example, in Examples 2 and 3). The claims recite a method in which a construct is introduced into a plant cell comprising a polynucleotide encoding an enzyme having organophosphate hydrolase activity, the cell when contacted with organophosphate hydrolyzes the organophosphate, and the hydrolysis detected such that extraction of the hydrolysis product and destruction of the cell is not necessary. Thus it is believed the section 102 rejection over Phillips et al. has been overcome, in that the reference discusses using organophosphate hydrolases to keep an insect alive.

Claims 1-40 were rejected under section 103 as obvious over Barrett in view of Jilka and Hood et al. Barrett was cited as teaching transformation of plant cells with a polynucleotide encoding P450 enzymes that metabolize organophosphates, which are used to protect plants from exposure to a pesticide. The Examiner notes Barrett does not teach transforming a plant cell with a nucleotide encoding an organophosphate hydrolase, and points to Jilka as showing a plant cell transformed with a polynucleotide encoding organophosphate hydrolase, and to Hood et al as using organophosphate paraoxon to detect OPH activity in a plant.

The Applicant points out that the claims of the present amendment teaching an invention that cannot be predictably produced using the teachings of the references. The claims recite a method for determining whether a cell has incorporated and expresses a polynucleotide using a construct comprising a polynucleotide encoding organophosphate

hydrolase activity, then contacting the cell or progeny with the organophosphate, then detecting hydrolysis without the necessity of destruction of the cell.

The Hood and Jilka references are cited as showing that organophosphate hydrolase encoding nucleotides can be expressed in cells, and in Hood, that the hydrolysis product can be detected spectrophotometrically after extraction of the tissue. However, there are many nucleotides in existence which encode proteins, and which further encode proteins, the expression of which can be detected by a variety of methods when extracted from the tissue. It is not predictable that such a nucleotide can be used as a marker, selectable or scorable, such that the tissue is not destroyed.

By way of example, many polynucleotides have been expressed in plants but relatively few are used as markers. This includes nucleotides that express proteins which can prevent cell killing with an herbicidal compound. For example, in US Patent 4,940,835 the development of a glyphosate-resistance plant cell is described and the construct with the nucleic acid encoding the resistance enzyme shown to be useful as a selectable marker. In the patent it is noted that a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme was known previously to catalyze the conversion of shikimate-3-phosphate into 5-enolpyruvyl-shikimate-3-phosphate, an intermediate in the biochemical pathway for creating three essential aromatic amino acids (tyrosine, phenylalanine, and tryptophan) and that glyphosate tolerant plant cells could be selected which overproduce EPSPS in the presence of low levels of glyphosate when plant cells were first selected with a different selectable marker. However, the inventors stated, in that prior work, none of the experiments demonstrated that such a method would be efficacious in differentiating plants. They went on to demonstrate the nucleic acids encoding the enzyme could be used as a selectable marker; an unpredictable development until their invention. Plants which produce a substance which can hydrolyze a chemical may or may not be useful as markers, in that the chemical to which it is exposed may overkill, thus providing no protection, or underkill, in which it is not possible to accurately select for those expressing the hydrolyzing compound. Thus, those working on the same polynucleotide may be able to express the protein in plants, but its usefulness as a marker is not predictable.

Even further attenuated is use of any particular protein as a marker in a manner in which destruction of the cell after hydrolyzizing a compound is not necessary in order to detect expression of the protein. As noted, many metabolizing proteins may be produced in a plant, but use a scorable marker is not predictable. Here, as with the beta glucuronidase (GUS) scorable marker, the organophosphate hydrolase protein is not one that is common in plants. Introduction of a sequence encoding the protein and providing the compounds for detection does not predict it will be successfully used as a visual marker not requiring the destruction of the plant cell. In US Patent 6,486,382 directed to the reporter marker for green fluorescent protein, the inventors discussed the fact that there are obstacles to overcome in identifying a scorable marker. They note the GUS gene typically requires destruction of plant tissue, and that *in vivo* means that do not require destruction have not proven useful for recovery of transformed cells because of low sensitivity and high fluorescent backgrounds. Thus, it still was not useful as a non-destructive marker, despite capability as a visual marker system. Further, even though green fluorescent protein had been successfully expressed in *Arabidopsis* cells and regenerated into whole plants, those plants exhibited signs of mild to moderate toxicity in the light compared to plants not expressing GFP, and the stronger expressing plant cells were difficult to regenerate. Thus, information suggesting that a protein may be expressed in a plant, and the expression detected, does not allow one skilled in this are art to conclude that it may be useful as a scorable marker. Thus it is believed the invention is not obvious.

Since the amendment does not enter any new matter and adopts the Examiner's proposals, and places the claims in condition for allowance, entry of the amendment is respectfully requested, and reconsideration and allowance of the claims.

Respectfully submitted,

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